Cyclic peptide derivatives as inhibitors of the integrin $\alpha_v\beta_6$

The invention relates to novel peptide derivatives of the formula I

cyclo(Arg-X^1-Asp-X^2-X^3-X^4-X^5-X^6-R^1) I

in which

X^1 is Ser, Gly or Thr,
X^2 is Leu, Ile, Nle, Val or Phe,
X^3 is Asp, Glu, Lys or Phe,
X^4 is Gly, Ala or Ser,
X^5 is Leu, Ile, Nle, Val or Phe,
X^6 is Arg, Har or Lys,
R^1 is absent or is one or more ω-aminocarboxylic acid residue(s), the ω-aminocarboxylic acid residue(s) having a length of 500 to 2500 pm,

where the amino acids mentioned can also be derivatized,
the D and the L forms of the optically active amino acid residues are included,
and their physiologically acceptable salts and solvates.

The invention was based on the object of finding novel compounds having valuable properties, in particular those which can be used for the production of medicaments.

It has been found that the compounds according to the invention and their salts have very valuable pharmacological properties together with good tolerability.
The peptides according to the invention can be employed as efficacious inhibitors of the $\alpha_v\beta_6$ integrin receptor

and thus for the treatment of various diseases and pathological findings.

Other inhibitors of the integrin $\alpha_v\beta_6$ are described in DE 19858857 and by S. Kraft et al. in J. Biol. Chem. 274, 1979-85 (1999). The compounds according to the invention are to be considered as a selection invention with respect to the application mentioned.

Integrins belong to the family of heterodimers of Class I - transmembrane receptors which play an important role in numerous cell-matrix or cell-cell adhesion processes (Tuckwell et al., 1996, Symp. Soc. Exp. Biol. 47). They can be roughly divided into three classes: the β_1 integrins, which are receptors for the extracellular matrix, the β_2 integrins, which are activatable on leucocytes and are "triggered" during inflammatory processes, and the α_v integrins, which influence the cell response during wound-healing and other pathological processes (Marshall and Hart, 1996, Semin. Cancer Biol. 7, 191).

The integrins $\alpha_5\beta_1$, $\alpha_{IIb}\beta_3$, $\alpha_8\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_8$ and $\alpha_v\beta_6$ all bind to the Arg-Gly-Asp (RGD) peptide sequence in natural ligands, such as, for example, fibronectin or vitronectin. Soluble RGD-containing peptides are able to inhibit the interaction of each of these integrins with the corresponding natural ligands. $\alpha_v\beta_6$ is a relatively rare integrin (Busk et al., 1992 J. Biol. Chem. 267(9), 5790), which is formed to an increased extent in repair processes in epithelial tissue and preferably binds the natural matrix molecules fibronectin and tenascin (Wang et al., 1996, Am. J. Respir. Cell Mol. Biol. 15(5), 664). The physiological and pathological functions of $\alpha_v\beta_6$ are still not precisely known; it is suspected, however, that this integrin plays an important role in physiological processes and disorders (e.g. inflammation, wound healing, tumours) in which

epithelial cells are involved. Thus $\alpha_v\beta_6$ is expressed on keratinocytes in wounds (Haapasalmi et al., 1996, J. Invest. Dermatol. 106(1), 42), from which it is to be assumed that in addition to wound-healing processes and inflammation, other pathological skin events, such as, for example, psoriasis, can also be influenced by agonists or antagonists of the said integrin. $\alpha_v\beta_6$ furthermore plays a role in the respiratory tract epithelium (Weinacker et al., 1995, Am. J. Respir. Cell Mol. Biol. 12(5), 547), so that appropriate agonists/antagonists of this integrin could be successfully employed in respiratory tract disorders, such as bronchitis, asthma, pulmonary fibrosis and respiratory tract tumours. Finally, it is known that $\alpha_v\beta_6$ also plays a role in the intestinal epithelium, so that appropriate integrin agonists/antagonists could be used in the treatment of inflammation, tumours and wounds of the stomach/intestinal tract.

The dependence of the formation of angiogenesis on the interaction between vascular integrins and extracellular matrix proteins is described by P.C. Brooks, R.A. Clark and D.A. Cheresh in Science 264, 569-71 (1994).

The object was therefore, in addition to the previously known natural high molecular weight ligands and antibodies, which are therapeutically and diagnostically difficult to handle, to find potent, specific and selective low molecular weight ligands for $\alpha_v\beta_6$, preferably peptides, which can be used for the therapeutic areas mentioned, but also as diagnostic or reagent.

It has been found that the peptide compounds according to the invention and their salts, as soluble molecules, exert an effect on cells which carry the said receptor, or, if they are bound to surfaces, are artificial ligands for $\alpha_v\beta_6$-mediated cell adhesion. Above all, they

act as $\alpha_v\beta_6$ integrin inhibitors, where they particularly inhibit the interactions of the receptor with other ligands, such as, for example, the binding of fibronectin. This action can be detected, for example, by the method which is described by J.W. Smith et al. in J. Biol. Chem. 265, 12267-12271 (1990).

It has furthermore been found that the novel substances have very valuable pharmacological properties together with good tolerability and can be employed as medicaments. This is described more precisely further below.

The peptide compounds according to the invention can furthermore be used *in vivo* and *in vitro* as diagnostics for the detection and localization of pathological conditions in the epithelial system, if they are equipped with appropriate markers (e.g. the biotinyl radical) according to the prior art.
The invention also comprises combinations with at least one other active compound and/or conjugates with other active compounds, such as cytotoxic active compounds and conjugates with radiolabels for X-ray therapy or PET diagnosis, but also fusion proteins with marker proteins such as GFP or antibodies, or therapeutic proteins such as IL-2.

Particularly active compounds are those of the formula I in which an octapeptide sequence cyclo(Arg-X^1-Asp-X^2-X^3-X^4-X^5-X^6), in which the radicals X^1, X^2, X^3, X^4, X^5 and X^6 have the meanings indicated, is expanded by R^1. The effect of the ring expansion is shown in Fig. 1 by the example of cyclo(Arg-Gly-Asp-Leu-D-Asp-Ala-Leu-Arg) [EMD 271588]. Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-NH_2 [EMD 271293] serves as a comparison compound.
Some cyclo(Arg-Gly-Asp-Leu-D-Asp-Ala-Leu-Arg-R^1) peptides, the distance from R^1 (calculated) and the value Q (IC_{50}[substance]/IC_{50}[EMD 271293]) and -log Q are given in the following table.

R^1	Distance [pm]	Q	-log Q
0	0	47	-1.672
Gly	370	2.1	-0.322
Abu	617	0.028	1.553
Gly-Gly	740	0.05	1.301
Aha	870	0.036	1.444
Aee	1078	0.038	1.420
Gly-Gly-Gly	1110	0.03	1.523
Abu-Abu	1235	0.03	1.523
Gly-Gly-Gly-Gly	1480	0.033	1.481
Aha-Aha	1740	0.036	1.444
Gly-Gly-Gly-Gly-Gly	1850	0.046	1.337
Gly-$(Gly)_4$-Gly	2220	0.05	1.301

The graphic representation can be seen in Fig. 1. Particularly active compounds are obtained even if the spacer length R^1 has reached approximately 500 pm.

Particularly preferred compounds of the formula I are those in which R^1 is one or more ω-aminocarboxylic acid residue(s), the ω-aminocarboxylic acid residue(s) having a length of 600 to 2500 pm, very particularly preferably those having a spacer length of 600 to 2000 pm.

ω-Aminocarboxylic acid residue(s) is/are understood as meaning any ω-aminocarboxylic acid, among which the amino acids below selected from the group consisting of Ala, Asn, Asp, Arg, Cys, Gln, Glu, Hcy, His, Hse, Ile, Leu, Lys, Met, Pen, Phe, Pro, Ser, Thr, Trp, Tyr, Val and $H_2N-(CH_2CH_2O)_m-(CH_2)_n-COOH$,

where m, n in each case independently of one another are 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12,

with the proviso that m + n is > 0,

are particularly preferred.

The invention thus preferably relates to peptide derivatives according to Claim 1, of the formula I

Cyclo(Arg-X^1-Asp-X^2-X^3-X^4-X^5-X^6-R^1) I

in which

X^1 is Ser, Gly or Thr,
X^2 is Leu, Ile, Nle, Val or Phe,
X^3 is Asp, Glu, Lys or Phe,
X^4 is Gly, Ala or Ser,
X^5 is Leu, Ile, Nle, Val or Phe,
X^6 is Arg, Har or Lys,
R^1 is absent or is 1-10 amino acids selected from the group consisting of Ala, Asn, Asp, Arg, Cys, Gln, Glu, Hcy, His, Hse, Ile, Leu, Lys, Met, Pen, Phe, Pro, Ser, Thr, Trp, Tyr, Val and $H_2N-(CH_2CH_2O)_m-(CH_2)_n-COOH$,
m,n in each case independently of one another are 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12, with the proviso that m + n is > 0,

where the amino acids mentioned can also be derivatized,
the D and the L forms of the optically active amino acid residues are included,
and their physiologically acceptable salts.

Some preferred groups of compounds can be expressed by the following subformulae Ia to Ih, which correspond to the formula I and in which the radicals not designated in greater detail have the meaning [sic] indicated in the formula I, but in which

in a) X^1 is Gly or Thr;

in b) X^1 is Gly or Thr,
X^2 is Leu;

in c) X^1 is Gly or Thr,
X^2 is Leu,
X^3 is Asp or D-Asp;

in d) X^1 is Gly or Thr,
X^2 is Leu,
X^3 is Asp or D-Asp,
X^4 is Gly or Ala;

in e) X^1 is Gly or Thr,
X^2 is Leu,
X^3 is Asp or D-Asp,
X^4 is Gly or Ala,
X^5 is Leu;

in f) X^1 is Gly or Thr,
X^2 is Leu,
X^3 is Asp or D-Asp,
X^4 is Gly, Ala or Ser,
X^5 is Leu,
X^6 is Arg;

in g) X^1 is Gly or Thr,
X^2 is Leu,
X^3 is Asp or D-Asp,
X^4 is Gly, Ala or Ser,
X^5 is Leu,
X^6 is Arg;
R^1 is 1-10 amino acids selected from the group consisting of Ala, Asn, Asp, Arg, Cys, Gln, Glu, Hcy, His, Hse, Ile, Leu, Lys, Met, Pen, Phe, Pro, Ser, Thr, Trp, Tyr, Val and $H_2N-(CH_2CH_2O)_m-(CH_2)_n-COOH$,
m,n in each case independently of one another are 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12,
with the proviso that m + n is > 0;

in h) X^1 is Gly or Thr,
X^2 is Leu,
X^3 is Asp or D-Asp,
X^4 is Gly, Ala or Ser,
X^5 is Leu,
X^6 is Arg;
R^1 is 1-6 amino acids selected from the group consisting of Gly, β-Ala, Abu or Aha;

and their salts.

The invention relates in particular to peptide compounds selected from the group consisting of

cyclo(Arg-Gly-Asp-Leu-Asp-Ala-Leu-Arg-Gly-Gly-Gly),
cyclo(Arg-Gly-Asp-Leu-Asp-Gly-Leu-Arg-Gly-Gly-Gly),
cyclo(Arg-Gly-Asp-Leu-D-Ala-Ala-Leu-Arg-Gly-Gly-Gly),
cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Gly-Gly-Gly),
cyclo(Arg-Gly-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu),
cyclo(Arg-Gly-Asp-Leu-D-Asp-Ala-Leu-Arg-Aha-Aha),
cyclo(Arg-Gly-Asp-Leu-D-Asp-Ala-Leu-Arg-Aha),
cyclo(Arg-Gly-Asp-Leu-D-Asp-Ala-Leu-Arg-Aee),
cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu),
cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-β-Ala),
cyclo(Arg-Gly-Asp-Leu-D-Asp-Ala-Leu-Arg-β-Ala),

and their physiologically acceptable salts.

The abbreviations of amino acid residues mentioned above and below stand for the radicals of the following amino acids:

Abu	4-aminobutyric acid
Aee	H_2N-$(CH_2CH_2O)_2$-CH_2COOH
Aha	6-aminohexanoic acid, 6-aminocaproic acid
Aib	α-aminoisobutyric acid
Ala	alanine
Asn	asparagine

Asp	aspartic acid
Arg	arginine
Bgl	C-alpha-tert-butylglycine
Cys	cysteine
Dab	2,4-diaminobutyric acid
Dap	2,3-diaminopropionic acid
Gln	glutamine
Glp	pyroglutamic acid
Glu	glutamic acid
Gly	glycine
Har	homoarginine
Hcy	homocysteine
His	histidine
homo-Phe	homophenylalanine
Hse	homoserine
Ile	isoleucine
Leu	leucine
Lys	lysine
Met	methionine
Nal	naphth-2-ylalanine
Nle	norleucine
Orn	ornithine
Pen	penicillamine
Phe	phenylalanine
Phg	phenylglycine
4-Hal-Phe	4-halophenylalanine
Pro	proline
Ser	serine
Thr	threonine
TIS	triisopropylsilane [sic]
Trp	tryptophan
Tyr	tyrosine
Val	valine.

In addition, the following have the meanings below:

Ac	acetyl
BOC	tert-butoxycarbonyl
BSA	bovine serum albumin

CBZ or Z	benzyloxycarbonyl
DCCl	dicyclohexylcarbodiimide
DCM	dichloromethane
DIEA	diethylamine
DMF	dimethylformamide
EDCl	N-ethyl-N,N'-(dimethylaminopropyl)-carbodiimide
Et	ethyl
FCA	fluoresceincarboxylic acid
FITC	fluorescein isothiocyanate
Fmoc	9-fluorenylmethoxycarbonyl
FTH	fluoresceinthiourea
HOBt	1-hydroxybenzotriazole
Me	methyl
MBHA	4-methylbenzhydrylamine
Mtr	4-methoxy-2,3,6-trimethylphenylsulfonyl
HONSu	N-hydroxysuccinimide
OBut	tert-butyl ester
Oct	octanoyl
OMe	methyl ester
OEt	ethyl ester
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
Pmc	2,2,5,7,8-pentamethylchroman-6-sulfonyl
POA	phenoxyacetyl
Sal	salicyloyl
TBS++	tris buffered saline with divalent cations
TBSA	TBS + BSA
TBTU	2-(1H-benzotriazol-1-yl)-1,1,3-tetramethyluronium tetrafluoroborate
TFA	trifluoroacetic acid
Trt	trityl (triphenylmethyl).

If the abovementioned amino acids can occur in two or more enantiomeric forms, all these forms and their mixtures (for example the DL forms) are included above and below. In addition, the amino acids can be provided with appropriate protective groups which are known per se.

The invention also relates to the hydrates and solvates, e.g. alcoholates, of these compounds.

So-called prodrug derivatives are included in the compounds according to the invention, that is compounds modified with, for example, alkyl or acyl groups, sugars or oligopeptides, which are cleaved rapidly in the body to give the active compounds according to the invention. These also include biodegradable polymer derivatives of the compounds according to the invention, as is described, for example, in Int. J. Pharm. 115, 61-67 (1995).

The amino acids and amino acid residues mentioned, such as, for example, the NH functions or terminal amide functions, can also be derivatized, the N-methyl, N-ethyl, N-propyl, N-benzyl or C_α-methyl derivatives being preferred. Derivatives which are additionally preferred are those of Asp and Glu, in particular the methyl, ethyl, propyl, butyl, tert-butyl, neopentyl or benzyl esters of the side chain carboxyl groups, and in addition also derivatives of Arg, which can be substituted on the $-NH-C(=NH)-NH_2$ group by an acetyl, benzoyl, methoxycarbonyl or ethoxycarbonyl radical.

In the compounds according to the invention, which are linked to one another in peptide fashion via the α-amino and α-carboxy groups (head-tail linkage), those cyclic compounds are also contained which, e.g. in the presence of a functional side chain, such as, for example, an SH group, are linked in the following way

side-side e.g. S-S (disulfide),
head-side
side-tail.

Furthermore, derivatives additionally included in the compounds according to the invention are those which

consist of the actual peptides according to the invention and known marker compounds which make it possible to detect the peptides easily. Examples of such derivatives are radiolabelled, biotinylated or fluorescence-labelled peptides.

A fluorescent dye radical is preferably 7-acetoxy-coumarin-3-yl, fluorescein-5-(and/or 6-)yl, 2',7'-dichlorofluorescein-5-(and 6-)yl, dihydrotetramethyl-rosamin-4-yl, tetramethylrhodamin-5-(and 6-)yl, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-ethyl or 4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-s-indacene-3-ethyl.

Suitable functionalized fluorescent dye radicals which can serve as reagents for the preparation of the compounds of the formula I according to the invention are described, for example, in "Handbook of Fluorescent Probes and Research Chemicals, 5th Edition, 1992-1994, by R.P. Haughland, Molecular Probes, Inc.".

The invention comprises not only the peptides mentioned but also mixtures and preparations which, in addition to these compounds according to the invention, also contain other pharmacological active compounds or adjuvants which can influence the primary pharmacological action of the peptides according to the invention in a desired manner.

The compounds according to the invention and also the starting substances for their preparation are otherwise prepared by methods which are known per se and frequently employed, such as are described in the literature (e.g. in the standard works such as Houben-Weyl, Methoden der organischen Chemie (Methods of Organic Chemistry), Georg-Thieme Verlag, Stuttgart), namely under reaction conditions which are known and suitable for the reactions mentioned. Use can also be made in this case of variants which are known per se.

Preferably, the peptides according to the invention can be prepared by means of solid-phase synthesis and subsequent removal and purification, as was described, for example, by *Jonczyk und Meienhofer* (Peptides, Proc. 8th Am. Pept. Symp., Eds. V. Hruby and D.H. Rich, Pierce Comp. III, pp. 73-77, 1983, or Angew. Chem. 104, 1992, 375) or according to Merrifield (J. Am. Chem. Soc. 94, 1972, 3102).

The peptides according to the invention can be prepared on a solid phase (manually or in an automated synthesizer) in an Fmoc strategy using acid-labile side protective groups and purified by means of RP-HPLC. The peak homogeneity can be measured by RP-HPLC and the substance identity by means of FAB-MS.

Otherwise, the peptides can be prepared by customary methods of amino acid and peptide synthesis, such as is known, for example, from Novabiochem - 1999 Catalog & Peptide Synthesis Handbook of Calbiochem-Novabiochem GmbH, D-65796 Bad Soden, from numerous standard works and published patent applications.

Stepwise couplings and fragment condensations can be utilized. Different N-terminal, C-terminal and side protective groups can be used, which are preferably selected to be orthogonally cleavable. Coupling steps can be carried out using different condensing reagents such as carbodiimides, carbodiimidazole, those of the uronium type such as TBTU, mixed anhydride methods, and acid halide or active ester methods. Activated esters are expediently formed in situ, for example by addition of HOBt or N-hydroxysuccinimide.

The cyclization of a linear precursor molecule having side protective groups can likewise be carried out using such condensation reactions, as is described, for example, in DE 43 10 643 or in Houben-Weyl, l.c., Volume 15/II, pages 1 to 806 (1974).

Different resins and anchor functions can be utilized in the solid-phase peptide synthesis. Resins can be based, for example, on polystyrene or polyacrylamide, anchor functions such as Wang, o-chlorotrityl are utilizable for the preparation of peptide acids, aminoxanthenoxy anchors, for example for the preparation of peptide amides.

Biotinylated or fluorescence-labelled peptides/proteins can likewise be prepared by standard methods (for example E.A. Bayer and M. Wilchek in Methods of Biochemical Analysis Vol. 26 The Use of the Avidin-Biotin Complex as a Tool in Molecular Biology; and Handbook of Fluorescent Probes and Research Chemicals, 6th Edition, 1996, by R.P. Haugland, Molecular Probes, Inc.; or alternatively *WO 97/14716*).

Of course, the peptides according to the invention can also be liberated by solvolysis, in particular hydrolysis, or by hydrogenolysis of their functional derivatives. Preferred starting substances for the solvolysis or hydrogenolysis are those which, instead of one or more free amino and/or hydroxyl groups, contain corresponding protected amino and/or hydroxyl groups, preferably those which, instead of an H atom which is connected to an N atom, carry an amino protective group or which, instead of the H atom of a hydroxyl group, carry a hydroxyl protective group. The same applies to carboxylic acids which can be protected by substitution of their -CO-OH hydroxyl function by means of a protective group, for example as an ester.

The expression "amino protective group" is generally known and relates to groups which are suitable for protecting (or blocking) an amino group from chemical reactions, but which are easily removable after the desired chemical reaction has been carried out at other positions in the molecule. The expression "hydroxyl protective group" is likewise generally known and

relates to groups which are suitable for protecting a hydroxyl group from chemical reactions, but which are easily removable after the desired chemical reaction has been carried out at other positions in the molecule. The liberation of the compounds from their functional derivatives takes place - depending on the protective group utilized - for example using strong acids, expediently using TFA or perchloric acid, but also using other strong inorganic acids such as hydrochloric acid or sulfuric acid, strong organic carboxylic acids such as trichloroacetic acid or sulfonic acids such as benzene- or p-toluenesulfonic acid. Hydrogenolytically removable protective groups (for example CBZ or benzyl) can be removed, for example, by treatment with hydrogen in the presence of a catalyst (for example of a noble metal catalyst such as palladium, expediently on a support such as carbon).

Typical protective groups for N termini and for side-position amino groups are Z, BOC, Fmoc, those for C-termini or the Asp or Glu side chains are O-prim-alkyl (for example OMe or OEt), O-tert-alkyl (for example OBut) or OBenzyl. Z, BOC, NO_2, Mtr, Pmc or Pbf, for example, is suitable for the guanidino function of the Arg. Alcoholic functions can be protected by benzyl radicals, tert-alkyl radicals or trityl groups.

The groups BOC, OBut and Mtr can preferably be removed, for example, using TFA in dichloromethane or using approximately 3 to 5 N HCl in dioxane at 15-30°, the FMOC [sic] group using an approximately 5 to 50% solution of dimethylamine, diethylamine or piperidine in DMF at 15-30°.

The trityl group is employed, for example, for the protection of the amino acids histidine, asparagine, glutamine and cysteine. Removal is carried out, depending on the desired final product, using TFA/10% thiophenol, the trityl group being removed from all

amino acids mentioned, when using TFA/anisole or TFA/thioanisole only the trityl group of His, Asn and Gln is removed, whereas that on the Cys side chain remains.

Hydrogenolytically removable protective groups (for example CBZ or benzyl) can be removed, for example, by treatment with hydrogen in the presence of a catalyst (for example of a noble metal catalyst such as palladium, expediently on a support such as carbon). Suitable solvents in this case are those indicated above, in particular, for example, alcohols such as methanol or ethanol or amides such as DMF. As a rule, the hydrogenolysis is carried out at temperatures between approximately 0 and 100° and pressures between approximately 1 and 200 bar, preferably at 20-30° and 1-10 bar. Hydrogenolysis of the CBZ group takes place readily, for example, on 5 to 10% Pd/C in methanol or using ammonium formate (instead of hydrogen) on Pd/C in methanol/DMF at 20-30°.

As already mentioned, the peptides according to the invention include their physiologically acceptable salts, which can likewise be prepared by standard methods. Thus, a base of a compound according to the invention can be converted into the associated acid addition salt using an acid, for example by reaction of equivalent amounts of the base and of the acid in an inert solvent such as ethanol and subsequent evaporation. Acids which are suitable for this reaction are in particular those which yield physiologically acceptable salts. Thus, inorganic acids can be used, for example sulfuric acid, nitric acid, hydrohalic acids such as hydrochloric acid or hydrobromic acid, phosphoric acids such as orthophosphoric acid, sulfamic acid, furthermore organic acids, in particular aliphatic, alicyclic, araliphatic, aromatic or heterocyclic mono- or polybasic carboxylic, sulfonic or sulfuric acids, e.g. formic acid, acetic acid,

propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, lactic acid, tartaric acid, malic acid, citric acid, gluconic acid, ascorbic acid, nicotinic acid, isonicotinic acid, methane- or ethanesulfonic acid, ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, naphthalenemono- and -disulfonic acids and laurylsulfuric acid. Salts with physiologically unacceptable acids, e.g. picrates, can be used for the isolation and/or purification of the compounds according to the invention. On the other hand, an acid of the compounds according to the invention can be converted into one of its physiologically acceptable metal or ammonium salts by reaction with a base. Possible salts in this case are in particular the sodium, potassium, magnesium, calcium and ammonium salts, furthermore substituted ammonium salts, for example the dimethyl-, diethyl- or diisopropylammonium salts, monoethanol-, diethanol- or diisopropylammonium salts, cyclohexyl- or dicyclohexylammonium salts, dibenzylethylenediammonium salts, furthermore, for example, salts with arginine or lysine.

The peptide compounds according to the invention can be employed, as already mentioned, as pharmaceutical active compounds in human and veterinary medicine, in particular for the prophylaxis and/or therapy of disorders in which epithelial cells are involved. Particularly to be emphasized in this context are disorders or inflammations or wound-healing processes of the skin, the respiratory tract organs and the stomach and intestinal region, thus, for example, apoplexy, angina pectoris, oncoses, osteolytic diseases such as osteoporosis, pathological angiogenic diseases such as, for example, inflammation, fibrosis, in particular pulmonary fibrosis, opthalmological diseases, diabetic retinopathy, macular degeneration,

myopia, ocular histoplasmosis, rheumatoid arthritis, osteoarthritis, rubeotic glaucoma, ulcerative colitis, Crohn's disease, atherosclerosis, psoriasis, restenosis after angioplasty, in acute kidney failure, nephritis, microbial infections and multiple sclerosis.

The invention accordingly relates to peptide compounds of the formulae defined above and below and in the claims including their physiologically acceptable salts as medicaments, diagnostics or reagents.

The invention in particular relates to appropriate medicaments as inhibitors for the control of disorders which are indirectly or directly based on expression of the $\alpha_v\beta_6$ integrin receptor, thus in particular in pathological angiogenic disorders, thromboses, cardiac infarct, coronary heart disorders, arteriosclerosis, tumours, osteoporosis, inflammation, infections and for influencing wound-healing processes.

[lacuna] also relates to appropriate pharmaceutical preparations, which contain at least one medicament of the formula I and, if appropriate, vehicles and/or excipients.

The invention furthermore relates to the use of the peptide compounds and/or their physiologically acceptable salts according to the claims and the description for producing a medicament for the control of disorders which are based indirectly or directly on expression of the $\alpha_v\beta_6$ integrin receptor, thus in particular in pathological angiogenic disorders, thromboses, cardiac infarct, coronary heart disorders, arteriosclerosis, tumours, osteoporosis, inflammation, infections and for influencing wound-healing processes.

The medicaments according to the invention or pharmaceutical preparations comprising them can be used in human or veterinary medicine. Possible vehicles are organic or inorganic substances which are suitable for enteral (e.g. oral) or parenteral administration, or

topical application or for administration in the form of an inhalation spray and do not react with the novel compounds, for example water, vegetable oils, benzyl alcohols, alkylene glycols, polyethylene glycols, glycerol triacetate, gelatin, carbohydrates such as lactose or starch, magnesium stearate, talc, petroleum jelly. In particular, tablets, pills, coated tablets, capsules, powders, granules, syrups, juices or drops are used for oral administration, suppositories are used for rectal administration, solutions, preferably oily or aqueous solutions, furthermore suspensions, emulsions or implants, are used for parenteral administration, and ointments, creams or powders are used for topical application. The novel compounds can also be lyophilized and the lyophilizates obtained can be used, for example, for the production of injection preparations. The preparations indicated can be sterilized and/or can contain excipients such as lubricants, preservatives, stabilizers and/or wetting agents, emulsifiers, salts for influencing the osmotic pressure, buffer substances, colorants, flavourings and/or [lacuna] more further active compounds, e.g. one or more vitamins.

For administration as an inhalation spray, sprays can be used which contain the active compound either in dissolved form or suspended in a propellant or propellant mixture (for example CO_2 or fluoro-chlorohydrocarbons). The active compound is expediently used in this case in micronized form, where one or more additional physiologically tolerable solvents can be present, e.g. ethanol. Inhalation solutions can be administered with the aid of customary inhalers.

As a rule, the substances according to the invention can be administered in analogy to other known, commercially available peptides (for example described in US-A-4 472 305), preferably in doses between approximately 0.05 and 500 mg, in particular between 0.5 and 100 mg, per dose unit. The daily dose is

preferably between approximately 0.01 and 20 mg/kg of body weight. The specific dose for each patient depends, however, on all sorts of factors, for example on the efficacy of the specific compound employed, on the age, body weight, general state of health, sex, on the diet, on the time and route of administration, and on the excretion rate, pharmaceutical combination and severity of the particular disorder to which the therapy relates. Parenteral administration is preferred.

Furthermore, the novel compounds of the formula I can be used in analytical biology and molecular biology.

The novel compounds of the formula I, where X is a fluorescent dye radical linked via a -CONH-, -COO-, -NH-C(=S)-NH-, -NH-C(=O)-NH-, $-SO_2NH-$ or -NHCO- bond, can be used as diagnostic markers in the FACS (**F**luorescence **A**ctivated **C**ell **S**orter) technique and fluorescence microscopy.

The use of labelled compounds in fluorescence microscopy is described, for example, by Y.-L. Wang and D.L. Taylor in "Fluorescence Microscopy of Living Cells in Culture, parts A + B, Academic Press, Inc. 1989".

The novel compounds according to the invention can also be used as integrin ligands for the preparation of columns for affinity chromatography for the preparation of integrins in pure form. The complex of an avidin-derivatized support material, e.g. Sepharose, and the novel compounds is formed by methods known per se (for example E.A. Bayer and M. Wilchek in Methods. of Biochemical Analysis Vol 26 The Use of the Avidin-Biotin Complex as a Tool in Molecular Biology). Suitable polymeric support materials here are the polymeric solid phases having preferably hydrophilic properties and known per se in peptide chemistry, for example crosslinked polysugars such as cellulose,

Sepharose or Sephadex®, acrylamides, polymers based on polyethylene glycol or Tentakel polymers®.

The invention finally also includes recombinant DNA sequences which contain sections which code for peptide regions which have the peptide structural motifs according to the invention.

DNA of this type can be transferred to cells by particles, as is described in Ch. Andree et al. Proc. Natl. Acad. Sci. 91, 12188-12192 (1994), or the transfer to cells can be increased by other excipients, such as liposomes (A.I. Aronsohn and J.A. Hughes J. Drug Targeting, 5, 163-169 (1997)).

The transfer of a DNA of this type could accordingly be utilized in yeasts, by means of baculoviruses or in mammalian cells for the production of the peptide substances of this invention.

If an animal or human body is infected with a recombinant DNA of this type, the peptides according to the invention finally themselves formed by the infected cells can immediately bind to the $\alpha_v\beta_6$ integrin receptor, for example of tumour cells, and block it. Corresponding recombinant DNA, which can be prepared by known and customary techniques, can, for example, however also be present in the form of virus DNA which contains sections which code for the virus coat protein. By infection of a host organism with recombinant, preferably non-pathogenic viruses of this type, host cells which express the integrin $\alpha_v\beta_6$ can preferably be attacked (targeting).

Suitable viruses are, for example, adenoviral types which have been used a number of times already as vectors for foreign genes in mammalian cells. A number of properties make them good candidates for gene therapy, as can be seen from S.J. Watkins et al. Gene

Therapy 4, 1004-1012 (1997) (see also J. Engelhardt et al. Hum. Gene Ther. 4, 759-769 (1993)).

As can be found in A. Fasbender et al. J. Clin. Invest. 102, 184-193 (1998), a common problem in gene therapy by means of viral and non-viral vectors is the limited efficiency of the gene transfer. Using the additional ligand sequence for $\alpha_v\beta_6$ integrin described above in the coat protein of the adenoviruses, an improvement in the transfer, for example, of cystic fibrosis transmembrane conductance regulator (CFTR) cDNA can be achieved.

In a manner similar to that in the work of T. Tanaka et al. Cancer Research 58, 3362-3369 (1998), instead of the DNA for angiostatin the DNA for the sequences of this invention can also be utilized for cell transfections by means of retroviral or adenoviral vectors.

The peptides according to the invention can also be employed within a liposome complex of lipid/peptide/DNA for a transfection of cell cultures together with a liposome complex consisting of lipid/DNA (without peptide) for use in gene therapy in man. The preparation of a liposome complex from lipid/DNA/peptide is described, for example, in Hart S.L. et al. 1998: Lipid-Mediated Enhancement of Transfection by a Non-Viral Integrin-Targeting Vector. Human Gene Therapy 9, 575-585.

A liposome complex of lipid/peptide/DNA can be prepared, for example, from the following stock solutions: 1 µg/µl of lipofectin (equimolar mixture of DOTMA (= N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl-ammonium chloride) and DOPE (dioleyl phosphatidyl-ethanolamine)), 10 µg/ml of plasmid DNA and 100 µg/ml of peptide. For this, both DNA and peptide are dissolved in cell culture medium.

The liposome complex is prepared by mixing the three components in a specific weight ratio (lipid:DNA:peptide, for example 0.75:1:4). Liposomal

DNA complexes for gene therapy in man have already been described (Caplen N.J. et al. 1995: Liposome-mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis Nature Medicine 1, 39-46).

The invention thus also relates to the use of accordingly modified recombinant DNA of gene-releasing systems, in particular virus DNA, for the control of diseases which are based indirectly or directly on expression of $\alpha_v\beta_6$ integrin receptors, thus in particular in pathological angiogenic disorders, thromboses, cardiac infarct, coronary heart disorders, arteriosclerosis, tumours, osteoporosis, inflammation, infections and for influencing wound-healing processes.

Above and below, all temperatures are indicated in °C.

The HPLC analyses (retention time Rt) were carried out in the following systems:
Column 5 µm LichroSpher 60 RP-Select B (250-4), with a 50-minute gradient from 0 to 80% 2-propanol in water/0.3% trifluoroacetic acid, at 1 ml/min flow and detection at 215 nm.

Mass spectrometry (MS): EI (electron impact ionization) M^+
FAB (fast atom bombardment) $(M+H)^+$

Example 1

Preparation and purification of peptides according to the invention:
In principle, the preparation and purification were carried out by means of Fmoc strategy with protection of acid-labile side chains on acid-labile resins using a commercially obtainable "continuous flow" peptide synthesizer according to Haubner et al. (J. Am. Chem. Soc. 118, 1996, 17703).

Cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu)
[EMD 272914]

2.7 g of Fmoc-Abu-OH (Bachem B-1910) was [sic] suspended in 150 ml of methylene chloride and dissolved until clear using 10 ml of DMF. 5.6 ml of diisopropylethylamine were added, the solution was poured onto 12.0 g of o-chlorotrityl chloride polystyrene resin (1.14 mmol/g, Bachem D-196512) and the batch was shaken at room temperature.
After 5 hours, the resin was filtered off with suction and washed with 300 ml each of DCM/MeOH/DIEA = 17/2/1, DCM, DMF, DCM and MeOH. After removal of the solvents, 14.55 g of Fmoc-amino acid resin were obtained. A triplicate Fmoc determination showed, on average, a loading of 541 µmol/g of Fmoc-Abu-O-oClTrt-resin.

0.7 g of Fmoc-Abu-O-oClTrt-polystyrene resin was successively subjected to a coupling step in a double-coupling technique 2 × with 0.30 g each of TBTU, 0.315 ml of ethyldiisopropylamine and Fmoc-amino acid in 4.1 ml of DMF in a commercial synthesis apparatus and a typical procedure (Milligen 9050 Pep Synthesizer™ apparatus and handbook, 1987), for 30 minutes in each case. Washing steps were carried out in DMF for 10 minutes, cleavage steps in piperidine/DMF (1:4 vol) for 5 minutes, and N-terminal acetylations (capping) were carried out with acetic anhydride/pyridine/DMF (2:3:15 vol) for 15 minutes.
The amino acids Fmoc-Arg(Pmc), then Fmoc-Leu, then Fmoc-Ala, then Fmoc-D-Asp(OBut), then Fmoc-Leu, then Fmoc-Asp(OBut), then Fmoc-Thr(But), then Fmoc-Arg(Pmc) and finally Fmoc-Abu were used. After cleavage of the Fmoc protective group from the Fmoc-Abu-Arg(Pmc)-Thr(But)-Asp(OBut)-Leu-D-Asp(OBut)-Ala-Leu-Arg(Pmc)-Abu-O-oClTrt-polystyrene resin, it was washed with DMF and isopropanol and, after drying in vacuo at room temperature, 0.9 g of Abu-Arg(Pmc)-Thr(But)-Asp(OBut)-

Leu-D-Asp(OBut)-Ala-Leu-Arg(Pmc)-Abu-O-oClTrt-polystyrene resin was obtained.

By treatment of this peptidyl resin with 20 ml of trifluoroethanol/dichloromethane/acetic acid (2:6:2 vol) for 2 hours at room temperature, filtration, concentration in vacuo and trituration with diethyl ether, 0.19 g of the side chain-protected peptide Abu-Arg(Pmc)-Thr(But)-Asp(OBut)-Leu-D-Asp(OBut)-Ala-Leu-Arg(Pmc)-Abu-OH was obtained.

By dropwise addition of a solution of this product in DMF (100 mg of peptide/15 ml of DMF) to a stirred solution of TBTU/HOBt/DIPEA (10:10:11 equivalents) in 50 ml of DMF/100 mg of peptide in the course of 30 minutes and further stirring for 1 hour, cyclization was achieved. After concentration and precipitation with water, 0.15 g of crude cyclo(Arg(Pmc)-Thr(But)-Asp(OBut)-Leu-D-Asp(OBut)-Ala-Leu-Arg(Pmc)-Abu-Abu) was obtained. After treatment with trifluoroacetic acid/water/TIS (94:3:3 vol) for 2 hours at room temperature, concentration in vacuo and trituration with diethyl ether, a precipitate of 85 mg of cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu) was obtained.

Purification of the product was carried out by RP-HPLC on Lichrosorb RP 18 (250 - 25, 7 µm, Merck KGaA) in 0.3% TFA using a gradient of 4% to 24% 2-propanol over one hour at 10 ml/min and assessment of the eluate by means of a UV flow-through photometer at 215 and 254 nm. 51 mg of product were obtained, FAB 1067; Rt [sic] 19.0.

The following products were prepared analogously:

Code (EMD)	Sequence	MW (g/mol)	FAB	Rt [sic]
271312	cyclo(RTDLDSLR)	957.1	958	15.10
271578	cyclo(RTDLdSLR)	957.1	957	17.51

Code (EMD)	Sequence	MW (g/mol)	FAB	Rt [sic]
271579	cyclo(RTDLdALR)	941.1	941	16.64
271586	cyclo(RGDLDSLR)	913.0	913	17.95
271587	cyclo(RGDLdSLR)	913.0	913	17.24
271588	cyclo(RGDLdALR)	897.0	897	19.60
271589	cyclo(RGDLdGLR)	882.9	883	16.68
271590	cyclo(RGDLd-β-Ala-LR)	897.0	897	15.47
271591	cyclo(RGDLdALRG)	954.1	954	16.79
271592	cyclo(RGDLdALRGG)	1011.1	1011	17.83
271593	cyclo(RGDLdALRGGG)	1068.2	1068	17.79
271594	cyclo(RGDLdALRGGGGGG)	1239.3	1239	17.30
272914	cyclo(RGDLdALR-Abu-Abu)	1067.2	1067	19.00
272966	cyclo(RTDLdALRGGG)	1112.2	1113	17.14
272967	cyclo(RTDLdGLRGGG)	1098.2	1099	19.00
272968	cyclo(RTDLDALRGGG)	1112.2	1113	17.39
272969	cyclo(RTDLDGLRGGG)	1098.2	1099	16.88
272970	cyclo(RGDLDALRG)	954.1	955	17.32
272971	cyclo(RaDLdALRGGG)	1082.2	1083	18.26
272972	cyclo(RGDLaALRGGG)	1082.2	1083	18.23
272958	cyclo(RGDLdALR-Aha-Aha)	1123.3	1123	19.38
272959	cyclo(RGDLdALR-Aha)	1010.2	1010	20.63
272960	cyclo(RGDLdALR-Aee)	1042.2	1042	18.92
272961	cyclo(RGDLdALRGGGG)	1125.2	1125	17.56
272962	cyclo(RGDLdALRGGGGG)	1182.3	1182	17.52
272963	cyclo(RGDLdGLRGGG)	1054.1	1054	15.59
272964	cyclo(RGDLDALRGGG)	1068.2	1069	16.84
272965	cyclo(RGDLDGLRGGG)	1054.1	1055	16.03
273028	cyclo(RTDLdALR-Aha)	1072.2	n.d.	19.30
273033	cyclo(RTDLdALR-Abu)	1044.2	n.d.	16.72
273035	cyclo(RGDLdALR-Abu)	1000.1	n.d.	16.96
273038	cyclo(RTDLdALR-Aha-Aha)	1185.4	n.d.	20.46
273040	cyclo(RTDLdALR-Abu-Abu) x 2 TFA	1111.3	n.d.	18.75
304219	cyclo(RTDLdALR-βAla)	1012.1	1012	19.4
304218	cyclo(RGDLdALR-βAla)	968.1	968	18.7
329400	cyclo(RGDLdALR)	811.9	812	21.15

Code (EMD)	Sequence	MW (g/mol)	FAB	Rt [sic]
329412	cyclo(RTDLdALR-Abu-Abu)	1111.3	1112	20.31
329402	cyclo(RGDLdALAGGG)	983.1	984	20.48
329399	cyclo(NMeArg-GDLaALRGGG)	1038.2	1039	19.66
329398	cyclo(R-NMeGly-DLaALRGGG)	1038.2	1039	19.76
326397	cyclo(RGD-NMeLeu-aALRGGG)	1038.2	1039	20.22
329396	cyclo(RGDL-[D-NMeAla]-ALRGGG)	1038.2	1039	20.55
329395	cyclo(RGDLa-NMeAla-LRGGG)	1038.2	1039	21.35
329394	cyclo(RGDLaA-NMeLeu-RGGG)	1038.2	1039	18.87
329393	cyclo(RGDLaAL-NMeArg-GGG)	1038.2	1039	20.64
	cyclo(RGDLdAAR)			
	cyclo(RGDLdAARGGG)			

Nomenclature for amino acids according to Eur. J. Biochem. 138, 9-37 (1984)
lower case letter = D-amino acid
n.d. = not determined

Example 2:

$\alpha_v\beta_6$ / fibronectin receptor binding test:

The prepared peptides according to the invention were bonded to the immobilized $\alpha_v\beta_6$ receptor together with competitively acting fibronectin in solution and the Q value was determined as a measure of the selectivity of the binding of the peptide to be tested to $\alpha_v\beta_6$. The Q value is calculated here from the quotient of the IC_{50} values of test peptide and a standard. The standard used was the linear Ac-RTDLDSLR-NH_2 (Code EMD 271293) (ref./patent cf. Pytela et al. Science 231, 1559,

(1986)). The binding test was carried out in detail as follows:

The immobilization of soluble $\alpha_v\beta_6$ receptor on microtitre plates was carried out by dilution of the protein solution in TBS++ and subsequent incubation overnight at 4°C (100 µl/hollow). Non-specific binding sites were blocked by incubation (2 h, 37°C) with 3% (w/v) BSA in TBS++ (200 µl/hollow). Excess BSA was removed by washing three times with TBSA++. Peptides were diluted serially (1:10) in TBSA++ and incubated with the immobilized integrin (50 µl of peptide + 50 µl of ligand per hollow; 2 h; 37°C) together with biotinylated fibronectin (2 µg/ml). Unbound fibronectin and peptides were removed by washing three times with TBSA++. The bound fibronectin was detected by incubation (1 h; 37°C) with an alkaline phosphatase-coupled anti-biotin antibody (Biorad) (1:20,000 in TBSA++; 100 µl/hollow). After washing three times with TBSA++, colorimetric detection was carried out by incubation (10-15 min; 25°C, in the dark) with substrate solution (5 mg of nitrophenyl phosphate, 1 ml of ethanolamine, 4 ml of H_2O; 100 µl/hollow). The enzyme reaction was stopped by addition of 0.4 M NaOH (100 µl/hollow). The colour intensity was determined at 405 nm in an ELISA measuring apparatus and made equal to the zero value. Hollows which were not coated with receptor served as a zero value. Ac-RTDLDSLR-NH_2 was employed as a standard. The IC_{50} values for the peptides tested were read off from a graph and from this, together with the IC_{50} value of the standard peptide, the Q value of the peptide according to the invention was determined.

Q value = IC_{50} test peptide / IC_{50} standard

Q values were calculated as means from repeat experiments.

The results of the test described are summarized in the Table 1 which follows:

Table 1

Results of the $\alpha_v\beta_6$ / fibronectin receptor binding test

Code (EMD)	Sequence	Q value = IC_{50} test peptide / IC_{50} EMD 271293
271293	Ac-RTDLDSLR-NH_2	1.00 (=75 nM)
271586	cyclo(RGDLDSLR)	26
271587	cyclo(RGDLdSLR)	88
271588	cyclo(RGDLdALR)	47
271589	cyclo(RGDLdGLR)	19
272970	cyclo(RGDLDALRG)	6.7
272964	cyclo(RGDLDALRGGG)	0.037
272965	cyclo(RGDLDGLRGGG)	0.16
272972	cyclo(RGDLaALRGGG)	0.05
271593	cyclo(RGDLdALRGGG)	0.03
272963	cyclo(RGDLdGLRGGG)	0.084
271590	cyclo(RGDLd-βAla-LR)	233
271591	cyclo(RGDLdALRG)	2.1
271592	cyclo(RGDLdALRGG)	0.05
271594	cyclo(RGDLdALRGGGGGG)	0.05
272914	cyclo(RGDLdALR-Abu-Abu)	0.03
272958	cyclo(RGDLdALR-Aha-Aha)	0.036
272959	cyclo(RGDLdALR-Aha)	0.036
272960	cyclo(RGDLdALR-Aee)	0.038
272961	cyclo(RGDLdALRGGGG)	0.033
272962	cyclo(RGDLdALRGGGGG)	0.046
273035	cyclo(RGDLdALR-Abu)	0.028
271312	cyclo(RTDLDSLR)	8
271578	cyclo(RTDLdSLR)	104
271579	cyclo(RTDLdALR)	12
272966	cyclo(RTDLdALR-GGG)	0.05
272967	cyclo(RTDLdGLR-GGG)	0.15
272968	cyclo(RTDLDALR-GGG)	0.14
272969	cyclo(RTDLDGLR-GGG)	0.18

Code (EMD)	Sequence	Q value = IC_{50} test peptide / IC_{50} EMD 271293
273028	cyclo(RTDLdALR-Aha)	0.015
273033	cyclo(RTDLdALR-Abu)	0.043
273038	cyclo(RTDLdALR-Aha-Aha)	0.015
273040	cyclo(RTDLdALR-Abu-Abu)	0.014
272971	cyclo(RaDLdALR-GGG)	8.2

The following examples relate to pharmaceutical preparations:

Example A: injection vials

A solution of 100 g of cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu) and 5 g of disodium hydrogenphosphate is adjusted to pH 6.5 in 3 l of double-distilled water using 2 N hydrochloric acid, sterile-filtered, dispensed into injection vials, lyophilized under sterile conditions and aseptically sealed. Each injection vial contains 5 mg of active compound.

Example B: suppositories

A mixture of 20 g of cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu) is fused with 100 g of soya lecithin and 1400 g of cocoa butter, poured into moulds and allowed to cool. Each suppository contains 20 mg of active compound.

Example C: solution

A solution is prepared from 1 g of cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu), 9.38 g of $NaH_2PO_4 \cdot 2\ H_2O$, 28.48 g of $Na_2HPO_4 \cdot 12\ H_2O$ and 0.1 g of benzalkonium chloride in 940 ml of double-distilled water. The mixture is adjusted to pH 6.8, made up to

1 l and sterilized by irradiation. This solution can be used in the form of eye drops.

Example D: ointment

500 mg of cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu) are mixed with 99.5 g of petroleum jelly under aseptic conditions.

Example E: tablets

A mixture of 1 kg of cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu), 4 kg of lactose, 1.2 kg of potato starch, 0.2 kg of talc and 0.1 kg of magnesium stearate is compressed in a customary manner to give tablets in such a way that each tablet contains 10 mg of active compound.

Example F: coated tablets

Analogously to Example E, tablets are pressed and are then coated in a customary manner with a coating of sucrose, potato starch, talc, tragacanth and colorant.

Example G: capsules

2 kg of cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu) are dispensed into hard gelatin capsules in a customary manner such that each capsule contains 20 mg of the active compound.

Example H: ampoules

A solution of 1 kg of cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu) in 60 l of double-distilled water is sterile-filtered, dispensed into ampoules, lyophilized under sterile conditions and aseptically sealed. Each ampoule contains 10 mg of active compound.

Example I: inhalation spray

14 g of cyclo(Arg-Thr-Asp-Leu-D-Asp-Ala-Leu-Arg-Abu-Abu) are dissolved in 10 l of isotonic NaCl solution and the solution is dispensed into commercially available spray containers having a pump mechanism. The solution can be sprayed into the mouth or nose. One puff of spray (approximately 0.1 ml) corresponds to a dose of approximately 0.14 mg.